

**EVALUATION OF TRANSGENIC COTTON:  
PRELIMINARY RESULTS OF THE COODETEC-  
CIRAD PROGRAM IN BRAZIL**

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**Abstract**

The objectives and first results of the cooperation program running between COODETEC and CIRAD are presented in this short note. Two species of the genus *Spodoptera* are being kept in the laboratory for evaluation of transformed lines of Coker 310 with protease inhibitor genes. Certain problems have been encountered in accustoming boll weevils to artificial diet. The adults did not lay eggs.

**Introduction**

The cotton pest complex in Brazil is very varied (Gondim et al., 1999). In Paraná state, *Anthonomus grandis* (Boheman) is considered as the main pest. In the central region where there has been considerable development of cotton in recent years (Mato Grosso), *Spodoptera frugiperda* (Smith), initially a maize pest, is often cited as cotton pest of economic importance. Other major pests are Coleoptera (*Eutinobothrus brasiliensis* (Hambleton), *Conotrachelus denieri* (Hustache)) Lepidoptera (tobacco budworm, bollworm, *Alabama argillacea* (Hubner)) and aphid vectors of viruses).

The CIRAD-COODETEC partnership has operated since 1990 and has recently developed new cotton varieties (CD401, CD402, CD403, CD404). In December 1998, the work of the partnership was extended to include cooperation in the evaluation of the transgenic lines bred by CIRAD in collaboration with the French national agricultural research institute INRA (*Institut National de Recherche Agronomique*) (Pannetier et al., 1997).

The objectives of the new research program are the evaluation of cotton germplasm (transformed lines) with regard to the pests mentioned above and future laboratory study of the potential use of bacterial toxins and other insecticidal substances before introduction in the plant.

**Materials and Methods**

The variety Coker 310 was transformed in France with genes of protease inhibitor OC-I, a cysteine-type protease inhibitor originally isolated from rice seeds, and C-II, a serine-type protease inhibitor isolated from soybean seeds.

The breeding methods tested for the insects mentioned above are the same as those used at the CIRAD laboratory in France for lepidopterans (Giret and Couilloud, 1986). The composition of the artificial diet used for the boll weevil is the same as that used at CIRAD (1670 ml water with 40 g agar, 2.4 g sorbic acid, 2 g nipagin, 100 g soybean flour, 10 g Wesson's salt®, 40 g Vanderzant vitamin mixture®, 20 g ascorbic acid, 60 g sugar, 60 g wheat germ, 40 g Pharmamedia® and 60 g yeast). Eggs are separated from the feed with 18% copper sulfate solution and sterilised with 0.3% MicroQuat® solution for 1 h 30 min.

The evaluation of transformed lines is somewhat complicated for boll weevil because of the biology of the pest and the action of the chemical (protease inhibitor) tested. Evaluation is based mainly on the work of McKibben and Villavaso (1997) and Greenplate et al. (1997) with observations on the development of larvae inside the square. Lepidopterans are being multiplied to achieve routine breeding sequences avoiding laboratory problems such as contamination by viruses. Many evaluation methods are cited in the literature and are still being tested in the laboratory. The test of leaf consumption by young caterpillars is the method chosen so far (based on that of Halcomb et al., 1996).

**Results and Discussion**

The team's first work has been that of establishing the breeding rooms, partially solving problems of temperature and humidity and obtaining permission from the national commission (CTNBio) to import the transformed lines. Because of the time taken to obtain material, and in particular Pharmamedia® powder from the USA for the preparation of the boll weevil diet, the field collection of insects began late in the season (March). Different breeding tests have been conducted.

The numbers of generations of each species of the genus *Spodoptera* obtained at the end of the first year of the program are shown in Table 1. The *Spodoptera* strains are still available. Other species, such as *Heliothis virescens* (F.), have been bred for four generations before dying at the adult stage without oviposition. The phylophagous species *A. argillacea* was collected as larvae in the field, but the adults did not lay eggs on the substrate.

Adult *A. grandis* were obtained from larvae fed on artificial diet. However, few eggs were laid by the adults, which seemed to prefer squares. We obtained only a single generation with few adults. In the current season, 188 adults

have been collected in the Palotina pheromone trap network. No eggs have yet been laid. The breeding of this species seems to necessitate a period of adaptation to the diet.

The evaluation tests on Lepidoptera will begin at the end of the construction of the special greenhouse in mid-February. Work on adaptation of the local strain of boll weevil to the conditions applied will continue and strains for the Mato Grosso will also be tested. The only Brazilian team that has succeeded in this type of breeding operation is that of the EMBRAPA/CENARGEN laboratory (Monnerat et al., 1999).

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Table 1. Species and origins of strains at the Coodetec laboratory in Cascavel.

Species	Location (State)	Host plant	Collection date	Number of generations obtained
<i>Spodoptera frugiperda</i>	Palotina (Paraná)	Maize	11 Mar 1999	8
	Primavera do Leste (Mato Grosso)	Maize	12 Apr 1999	7
<i>Spodoptera cosmioides</i>	Primavera do Leste (Mato Grosso)	Cotton	12 Apr 1999	6